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13. ABSTRACT (Maximum 200) The Breast Tissue Repository at Duke enters its fourth year of funding. The purpose of the Repository at Duke is to provide substantial quantities of frozen tissue for explorative molecular studies. The scope of our resource includes: 1.) freshly frozen tumor tissue from the primary and metastatic sites (lymph nodes), 2.) serum and plasma from patients and controls and, 3.) white blood cells (WBC's) from patients and controls. During the past year, tumor tissue from 76 breast cancer patients was acquired and banked. Sera, plasma and white blood cells from 157 patients were collected prior to surgery. The bank continued to acquire post-operative specimens. The US Army funding was used to collect blood from people in families with breast cancer participating in an NCI-sponsored clinical trial of counseling and genetic testing. Material (blood and tumor elements) was distributed to 16 separate laboratories, including five laboratories in institutions other than Duke.					
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FOREWORD

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Pin J. Subart 10/7/97
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INTRODUCTION

The Breast Cancer Tissue Repository at Duke University Medical Center is a US Army-funded infrastructure project. The coming year will be the fourth and final year of funding. During the life of this grant, we have banked tumor tissue from more than 250 patients. Frequently, primary cancer specimens and metastatic nodal tissue was retrieved from the same patient. In addition to tumor tissue, we have concentrated on collecting a bank of plasma and sera from patients taken prior to and after complete surgical extirpation. In this fashion, we have paired samples of blood from the same patient bearing a tumor and after removal of the vast majority of cancer. This resource may be helpful to investigators who are evaluating circulating tumor markers; a useful marker should change when measured before and after removal of the cancer. At the time of this report, we have a bank of blood samples from 400 patients.

The philosophy of our resource is unique. We encourage rapid use of our tissues for exploratory and descriptive projects. The Duke Repository is not designed for long-term prognostic studies involving keeping tissues for long periods and carefully monitoring their use. We collect large amounts of fresh-frozen material and require only a brief justification for its use. As the list of users indicates, all are highly qualified investigators with peer-reviewed research funding. Tissue from this Repository was used to identify BRCA1 and BRCA2 and to demonstrate the lack of mutations in these genes in sporadic breast cancer. Tissue from our Repository was used to demonstrate that the IGF-2 receptor acts as a tumor suppressor gene in breast cancer. Serum from Duke was used to verify the high percentage of breast cancer patients who circulate autoantibodies to normal and oncogenic cellular products.

Our Repository emphasizes flexibility to serve the requirements of investigators at our own institution. We collected sterile tumor from more than 100 patients for the isolation of tumor infiltrating lymphocytes (TIL's). This was done prospectively for Dr. Kent Weinhold at Duke working on a funded project within the Duke SPORE in Breast Cancer. The Duke SPORE sponsors a trial of counseling and testing for the BRCA1 and BRCA2 susceptibility genes. The US Army Repository personnel drew blood for this trial and now the Repository is helping Dr. Andrew Futreal at Duke ascertain and acquire blood from family members of patients testing negatively for BRCA1/2. This protocol is covered under separate IRB approval; the project uses the infrastructure of collection and distribution provided by the Repository.

BODY: Report on activities for the 1996-1997 year

During the past year, the tissue repository has continued to collect large volumes of tumor tissue. In each case, two or more blocks of tissue are frozen in OCT embedding medium. Residual tissue is flash frozen in liquid nitrogen and stored in air-tight vials. This year, we collected tissue from 86 patients. Of these, 18 included tissue from metastatic nodes in addition to the primary site in the breast. A number of cancers were collected fresh for isolation of tumor infiltrating lymphocytes (TILs) by Dr. Kent Weinhold. In this case, the patient was identified pre-operatively and arrangements made to harvest a portion of the tumor in a fresh and sterile fashion. This procedure is done in the surgical pathology laboratory adjacent to the operating rooms and under the supervision of an attending pathologist. Over the past three years of funding, we have collected a bank of TIL's from more than 100 tumors.

This year the bank provided fresh and sterile tumor tissue for attempts at continuous culture. Six cases were attempted and work is still in progress. Establishing continuous and immortal cell lines from breast cancer is a difficult process and success is infrequent. However, we would like to expand the number of breast cancer lines and animal xenografts available for research use. In the case of our Repository, we concentrated on cancers from patients with strong family histories of breast cancer.

Our blood bank has grown to substantial proportions. We now have blood and WBC's from 400 patients. This year, we added blood from 157 patients to the collection in addition to 84 follow-up blood draws from post-operative patients. For the initial blood draw, we obtain heparinized plasma, a clotted red top tube and a purple-top tube for collection of WBC's. Additional tubes have been added for special protocols; our IRB consent covers the collection of 30ml of blood or about five small tubes. This year, we collected normal WBC's from 135 additional subjects for establishing a bank of normal DNA's. These normal DNA's will serve as a reference for unique mutations in BRCA1 or BRCA2 found during genetic testing in a NCI-funded trial of testing and counseling. Finally, the Breast Repository phlebotomist has been used to obtain blood from family members of kindreds which segregate breast cancer but test negative for BRCA1/2 in the counseling trial. Thus far, our personnel have drawn blood from members of 12 such extended families or have arranged to have the blood drawn and sent to us.

Use of the bank has been brisk. The following table summarizes usage of the US Army-funded Breast Tissue Repository at Duke:

Table 1. Usage of the Duke Repository during 1996-1997

Investigator	Institution	Tissue Type	Quantity used
P. Andrew Futreal	Duke	Tumor	16
R. Jirtle	Duke	Tumor	25
A. White & C. Barrett	NIEHS	Tumor	150
L. Hale	Duke	Tumor	20
M. Dewhirst	Duke	Tumor	12
R. Young	U. South Carolina	Tumor	20
L. Harris	Duke	Tumor	12
S. Sukumar	Johns Hopkins	Tumor	176
A.B. Frey	NYU	Tumor	35
R. Ochs	Scripps Institute	Serum	110
P. Andrew Futreal	Duke	WBC's	135
K. Weinhold	Duke	WBC's	29
K. Weinhold	Duke	Tumor	20
A. Miron	Duke	WBC's	135
J. Marks	Duke	Tumor	250
C. Greenberg	Duke	Plasma	19

Prior to distributing tissue to our users, the Repository routinely cuts several frozen sections from the block of tissue to be used. The sections are fixed and stained with Diff-Quick and examined for the presence of tumor tissue. In certain cases, we have performed routine immunohistochemistry for common antigens such as p53, Ki-67, ER or PR, erbB-2, etc. This service is performed for users on a case-by-case basis. In other cases, we have macro-dissected frozen blocks to maximize the amount of tumor in relation to normal stroma. These extra services are consistent with our goal to provide quality tissue for exploratory biochemical and molecular experiments.

In the coming year, the Repository will continue to serve expanding research at Duke and within our network of collaborators in other institutions. The Duke SPORE received approval for a supplement to purchase a Laser Capture Microdissection Apparatus. The Department of Pathology at Duke is recruiting an experimental pathologist to work with the Breast Program at Duke who has a particular interest in microdissection. An additional supplement from the NCI has been approved to prepare cDNA libraries and representative PCR-based libraries of genomic DNA from ductal carcinoma in-situ and other stages of breast cancer progression. These libraries will be contributed to the national Genome Anatomy Project at the NIH. These projects will depend heavily on the tissue already collected within our Repository. The National Institutes of Environmental Health Sciences (NIEHS) has announced its Environmental Genome Initiative. Duke investigators will contribute to this effort and the Repository is expected to provide tumor and normal tissue for exploratory studies in this program. Finally, our genetic research program will continue family-based studies to discover new susceptibility genes and factors which modify risk in families segregating BRCA1 and BRCA2 mutations. The U.S. Army-funded Repository at Duke will continue to contribute to these efforts during the upcoming year and beyond.

CONCLUSIONS

The Breast Tissue Repository at Duke is an integral part of the Duke Comprehensive Cancer Center, The Duke SPORE in Breast Cancer and the Breast Program. The Repository has served research at Duke leading to important discoveries noted above. In the future, several important research initiatives at Duke, from the Department of Defense and from the NIH will be served by the Army-funded Repository. We expect to contribute tissue and blood to these and other efforts for years beyond the current funding period.